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A COMPARATIVE ANALYSIS OF NEUTRACEUTICAL AND THERAPEUTICAL POSSESSIONS OF *COSCINIUM FENESTRATUM* AND *MUKIA MADERASPATANA*

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ABSTRACT

The herbal plants are composed of a lot of active phytochemicals against pathogens, free radicals and hyperglycemia which provide an alternative means of therapy for various illnesses. The present study is designed to evaluate the biological properties of a polyphenol-rich fraction of the stem of *Coscinium fenestratum* and the leaves of *Mukia maderaspatana*. The antiradical activity of extracts was evaluated and the inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in a polyphenol-rich fraction of *Coscinium fenestratum* 74.16% and *Mukia maderaspatana* 71.29%. The antioxidant activity of extracts showed a strong positive correlation with the total phenolic content of the extract. The antibacterial effect of extracts showed more inhibitory activities against *Escherichia coli, Pseudomonas aeruginosa* and moderate action on *Staphylococcus aureus, Enterococcus faecalis*. The crude extracts had a lower level of sugars induced by amylase, glucosidase, and glucose absorption activities have inhibitory effects (EC50). As a consequence, their biochemical inhibitory impact might have been due to a synergistic impact among the phenolic chemicals present in the extract.

Keywords: Phytochemical, Polyphenol, Hyperglycemia, Antioxidant, Antibacterial

INTRODUCTION

Many traditional plants have medicinal properties due to the presence of natural antioxidants, particularly phenolic compounds **[1]**. These chemicals can scavenge reactive oxygen species, which can cause oxidative stress-related illnesses like cancer, hypertension, and neurological damage. A nutraceutical is a substance that can be classified as a food or a component of food that has medical or health advantages, including disease prevention and treatment. Because of their putative safety and possible nutritional and therapeutic advantages, plant-derived nutraceuticals have gained a lot of interest **[2]**.

Dietary fiber, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants, and other herbal/natural foods are examples of food products utilized as nutraceuticals **[3]**. These nutraceuticals aid in the treatment of some of the century's most pressing health issues, including obesity, cardiovascular disease, cancer, osteoporosis, arthritis, diabetes, and cholesterol. Overall, the term "nutraceutical" has ushered in a new era of medicine and health, in which the food business has evolved into a research-driven business **[4]**. Based on experimental tests, the current research aims to gain a deeper knowledge of the Phyto-nutraceuticals found in *Coscinium fenestratum* and *Mukia maderaspatana* plants.

MATERIALS AND METHODS

A botanist permitted the collection of *Coscinium fenestratum* stems and *Mukia maderaspatana* leaves from a local market. A blender was used to smash 100 g of *Coscinium fenestratum* pulp and turn it into a paste-like consistency. The moisture content of the homogenized sample was eliminated by freeze-drying it to obtain a powder. The powder form of both preparations was soaked in n-Hexane for 24 hours and then in methanol for 72 hours to produce methanol crude extract, which was concentrated using a rotatory evaporator at 40 degrees Celsius. The sticky residues were separated into chloroform soluble fractions and non-chloroform soluble fractions using chloroform, and the polyphenol-rich fraction was then dried in an oven.

Determination of total phenolic content:

The methanol extract (1 mL, 1 mg/mL) was carefully combined with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na2CO3, then centrifuged for 5 minutes at 13400X g. After 30 minutes of incubation at room temperature, the absorbance of the upper phase was measured using a UV–Vis Spectrophotometer (ELICO (SL150) at 750 nm **[5]**.

Estimation of flavonoid:

1 mL polyphenol-rich fraction of *Coscinium fenestratum* stem and *Mukia maderaspatana* leaves was completely combined with 1 mL 2 percent aluminum chloride and 0.5 mL 33 percent acetic acid, then 90 percent methanol was added and the mixture was thoroughly agitated for 30 minutes. At 414 nm, the absorbance was measured.

Thin-layer chromatography:

Thin layer chromatography of fractions was carried out according to established protocols. Using a microcapillary tube, the polyphenol-rich fraction was carefully put in pre-coated aluminum silica gel at 60 F. After allowing the spots to dry for a few minutes, the TLC plate was immersed in either a solvent mixture of Toluene, Acetone, and Formic acid (6:6:1) or solvents of ethyl acetate, glacial acetic acid, formic acid, and water (100:11:11:26 v/v/v/v). After drying, the TLC plates were examined and the RF value was calculated by using the standard formula.

ABTS (2,2'- Azino-bis-3-ethyl Benzthiazoline-6-sulphonic corrosive) radical scavenging assay:

ABTS extremist searching action items done by the technique depicted Re *et al*. (1999). ABTS extremist was newly set up from 5 ml of 4.9 mM potassium persulfate arrangement and 5 ml of 14 mM ABTS arrangement placed in dim for 16hours. It was then weakened with refined water to deliver an absorbance of 0.70 at 734 nm and was utilized for the cancer prevention agent action. For standard, 1 ml combination with 950 µl of ABTS arrangement and 50 µl of ascorbic acid was made. Correspondingly, for test tests, 1 ml of this blend with various centralization of each concentrate was taken. The response blend was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against refined water by utilizing a Deep Vision (1371) UV–Vis Spectrophotometer and contrasted and the control ABTS arrangement. Ascorbic acid was utilized as a reference antioxidant agent compound.

ABTS Scavenging Effect (%) = $[(A0-A1/A0) \times 100]$

Where A0 is the absorbance of the control response, A1 is the absorbance of concentrate.

Inhibiting lipid peroxidation activity:

The experimental mixture was prepared with 0.1 ml of egg yolk (25 percent w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO4 (NH4)2SO4.7H2O (0.06 mM); and various concentrations of the polyphenol-rich fraction of *Coscinium fenestratum* stem and *Mukia maderaspatana* leaves and incubated at 37°C for 1 hour. A total of 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%), 1.5 ml thiobarbituric acid (TBA) (0.8%), 1.5 ml acetic acid (0.8%) and pH 3.5. With distilled water, the final amount was increased to 4.0 ml, which was then held in a water bath at 95 to 100 °C for 1 hour. After cooling, the reaction mixture was added with 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v), shaken violently, and centrifuged at 4000 rpm for 10 minutes. To measure TBARS, the absorbance of the butanol-pyridine layer was measured at 532 nm in a Deep Vision (1371) UV–Vis Spectrophotometer. As a control, ascorbic acid was employed.

The percent inhibition of lipid peroxidation by each extract was estimated using 1-(E/C) 100.

Where C is the fully oxidized control's absorbance value and E is the absorbance of the test sample.

Superoxide radical scavenging activity:

The potential of the polyphenol-rich fraction of *Coscinium fenestratum* and *Mukia maderaspatana* to block the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT combination was used to develop this test **[6]**. Each 3 ml reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 mM riboflavin, 100 mM EDTA, 75 mM NBT, and various extract concentrations. It was kept visible in fluorescent light for 6 minutes, and absorbance was measured at 560 nm with a Deep Vision (1371) UV–Vis Spectrophotometer after that. As blanks, identical tubes with reaction mixture were kept in the dark. By comparing the absorbance of the control and test sample solutions, the percentage suppression of superoxide radical activity was calculated:

[(A0-A1)/A0] 100 percent superoxide radical scavenging capability

Where A0 represents control absorbance and A1 represents the absorbance of both plant extract fractions.

Metal chelating activity:

According to Iihami *et al*. **[7]** the metal-chelating capability of extracts was tested. 1 ml of various flavonoid-rich fraction concentrations was mixed with 0.05 ml of 2 mM ferric chloride solution. The reaction was started by adding 0.2 ml of 5 mM Ferrozine to the liquid and vigorously shaking it. The absorbance was measured at 562 nm against a blank after 10 minutes. Ascorbic acid was employed as a standard and all measurements were taken in triplicate. The following equation was used to compute the percent inhibition of the ferrozoine-Fe2+ combination.

$$\textbf{\% Inhibition of ferrozoine-Fe}^{2+}\textbf{ complex} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the flavonoid-rich fraction.

Nitric oxide radical scavenging activity:

The ability of the product to scavenge nitric oxide was determined using the method published by Olabinri *et al,* **[8]**. The extracts were combined with 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) and incubated at room temperature for 150 minutes. 0.2 ml Griess reagent (1 percent Sulfanilamide, 2 percent Phosphoric acid, and 0.1 percent N-(1-Naphthyl) eluted after the treatment period. At 546 nm, the absorbance of the experimental sample was measured against a blank. Ascorbic acid was employed as a standard and all measurements were taken in triplicate. The following equation was used to compute the percentage of inhibition:

% Nitric oxide radical scavenging capacity= $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the flavonoid-rich fraction.

Uptake of glucose by yeast cells:

Repeated centrifugation (3,000g, 5 min) of commercial baker's yeast in distilled water until clear supernatant fluids were recovered and a 10% (v/v) suspension was made in distilled water. Plant extracts in various concentrations (5-20g/mL) were added to 1mL of glucose solution (5, 10, 15, and 25 mM) and incubated for 10 minutes at 37 °C. The reaction was begun by adding 100 microliters of yeast suspension, vortexing it, and then incubating it at 37 °C for 60 minutes. Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Inhibition of α-amylase activity:

Alpha-amylase is an enzyme that breaks down the alpha bonds in big alpha-linked polysaccharides like glycogen and starch to produce glucose and maltose. The starch iodine method was first established by Kalita *et al*. **[9]** and afterward used by others for determining amylase activity in plant extracts with some modifications, was used to determine alpha-amylase inhibitory activity. 1 ml potato starch (1 percent w/v), 1 ml methanol extract and ethyl acetate of various concentrations such as 25, 50, 75, and 100 g/ml, 1 ml alpha-amylase enzyme (1 percent w/v), and 2 ml acetate buffer (0.1 M, 7.2 pH) were added to the alpha-amylase inhibition method. In an acetate buffer (820.3 mg sodium acetate and 18.7 mg sodium chloride), potato starch solution, alpha-amylase solution, and medication solution were produced.

Inhibitory activity against glycosidase:

The inhibitory activity of β-glucosidase was determined using a modified version of the usual approach **[10, 11]**. In a test tube, a volume of 25, 50, 75, and 100 g/ml of sample solution was incubated at 37ºC for 20 minutes with 50 l of 0.1 M phosphate buffer (pH 6.8) containing β-glucosidase solution (0.2 U/ml). After pre-incubation, each well was filled with 50µl of 5 mM p-nitrophenyl—D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) and incubated for additional 20 minutes at 37ºC. The reaction was then halted by adding 160µl of 0.2 M $NaCO_3$ to each well, and absorbance readings (A) were taken at 405 nm using a microplate reader and compared to a control that had 60µl of buffer solution. The enzyme solution was replaced with buffer solution for blank incubation (to allow for absorbance produced by the extract) and the absorbance was recorded. Inhibition percent was used to measure the inhibitory activity of β-glucosidase.

Antibiogram Assay:

The antibacterial effect of the extract was tested by using disc diffusion and minimum inhibitory concentration (MIC) techniques with control against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (**ATCC** 27853), *Escherichia coli* (**ATCC** 25922), and *Enterococcus faecalis* (ATCC 29212**)**. After 24 hours of incubation plates and tubes at 37°C, the results were read.

RESULTS AND DISCUSSION

The polyphenol-rich extract of the *Coscinium fenestratum* and *Mukia maderaspatana* were done and the yield of extract was found to be 51.1±1.4 (g/100 g). The total phenolic content was found to be 98.1±1.27 (mg catechin equivalents per gram ethyl acetate extract) and the total flavonoid content found to be 63.12±12 (mg catechin equivalents per gram ethyl acetate extract). Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis. In the partial characterization of polyphenol-rich extract done by TLC, the visible Rf value was calculated as 0.64 -0.68 and 62-65 respectively **(Figure 1)**.

The Free radical-scavenging ability of extracts was performed in different concentrations by using an ABTS assay and the results were tabulated **(Table 1)**. Results are expressed as a percentage inhibit of ABTS ability concerning to control. Each value represents the mean+SD of three experiments. The median effective concentration (EC50) found to be 65.23 in *Coscinium fenestratum* and 72.56 in *Mukia maderaspatana.*

Other biological screenings like inhibition of lipid peroxidation activity, Superoxide radical scavenging activity, metal chelating activity, nitric oxide radical scavenging activity, glucose uptake in yeast cells, α-amylase inhibition activity β-glucosidase inhibitory activity of extracts were carried out by using the concentration ranging 25µl/ml to 100µl/ml by absorbance in UV-Visible spectrophotometer. Results are expressed as percentage inhibition

(EC50) with concerning control and are listed in **Table 2**. Each value represents the mean+SD of three experiments. Overall, polyphenol-rich fractions of *Coscinium fenestratum* and *Mukia maderaspatana* yielded higher biological functional results when extract concentrations were measured.

Antimicrobial screening of extracts done by disc diffusion and MIC. Inhibitory zone size and amount of inhibiting growth versus concentration were observed **(Figure 2)** by including the 6.0 mm size of the well zone using a caliper. All the assays were duplicated, and the mean values were recorded in **Table 3**, and the graph **(Figure 3)** was drawn. According to the findings, the zones of inhibition in *Coscinium fenestratum* extract and *Mukia maderaspatana* were 17mm and 15.6mm at 100 l/ ml concentrations, respectively. Growth inhibition was seen in the MIC pathogenic strains concerning extract concentrations. Overall, *Escherichia coli* and *Pseudomonas aeruginosa* strains were shown to be more sensitive than the other two.

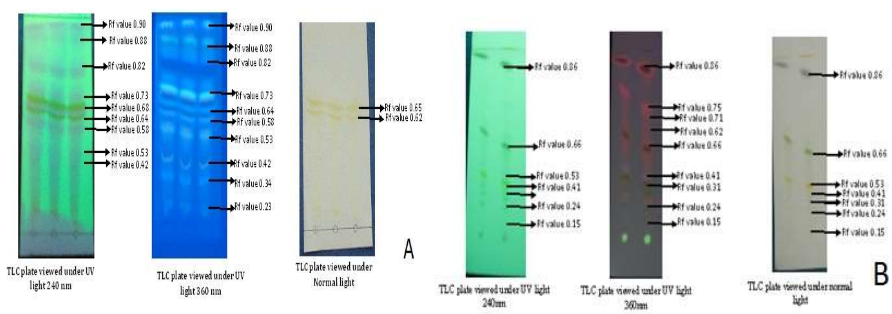


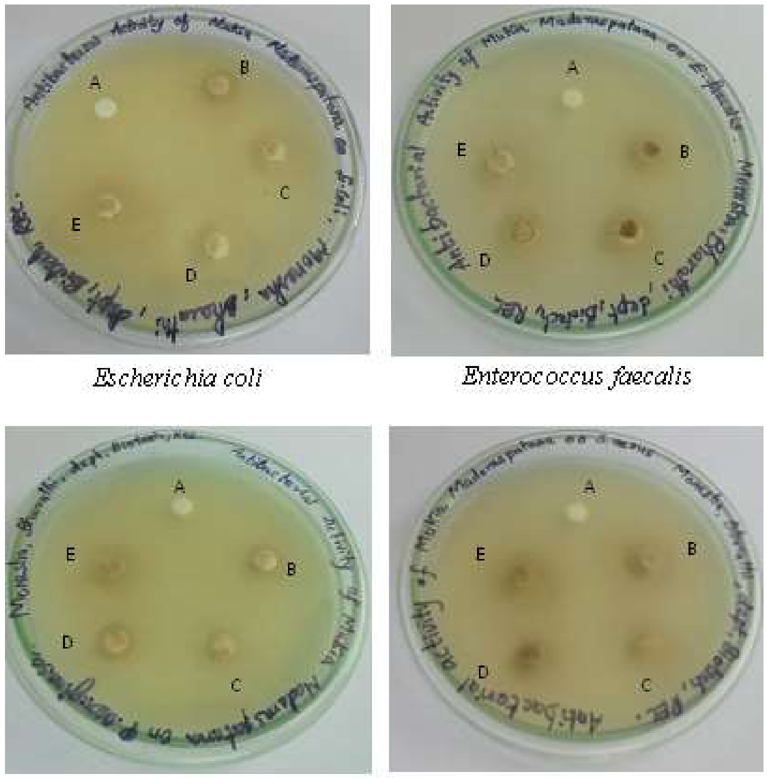
Figure 1: The Partial characterization of polyphenol rich fraction of stem of *Coscinium fenestratum* (A) and *Mukia maderaspatana* (B) by TLC

Table 1: ABTS radical activity *Coscinium fenestratum* and *Mukia maderaspatana*

Different concentration of extract	Polyphenol rich fraction of *Coscinium fenestratum*	Polyphenol rich fraction of *Mukia maderaspatana*	Standard Vitamin-C
25 µl/ml	25.85 ± 0.730	21.12 ± 0.334	19.59 ± 0.791
50 µl/ml	44.62 ± 0.626	40.59 ± 2.192	40.30 ± 0.964
75 µl/ml	63.47 ± 0.520	58.57 ± 1.155	55.45 ± 0.349
100 µl/ml	74.16 ± 0.866	71.79 ± 0.567	70.04 ± 0.957
EC_{50} value	65.23	72.56	78.96

Table 2: EC50 findings of different experiments

S. No.	Test	*Coscinium fenestratum* (EC_{50} value)	*Mukia maderaspatana* (EC_{50} value)
1.	Inhibition of lipid peroxidation activity	69.23	102.56
2.	Superoxide radical scavenging activity	73.26	82.16
3.	Metal chelating activity	69.46	81.23
4.	Nitric oxide radical scavenging activity	61.49	93.12
5.	Glucose uptake in yeast cells	65.23	102.46
6.	α-Amylase inhibition activity	69.18	98.12
7.	β-Glucosidase inhibitory activity	86.12	92.35



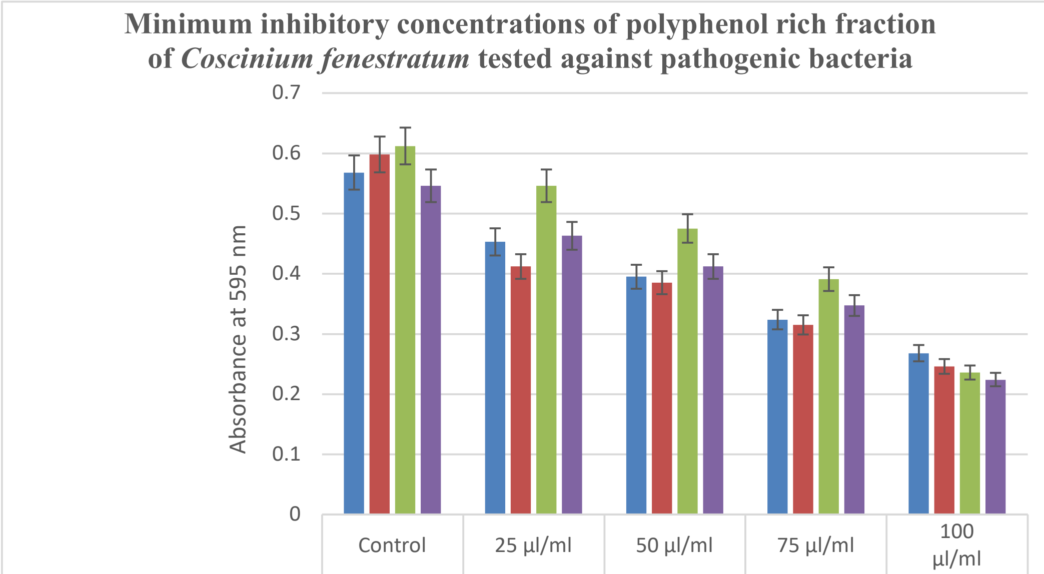
Escherichia coli *Enterococcus faecalis*

Pseudomonas aeruginosa *Staphylococcus aureus*

Figure 2: The antibacterial activity of the polyphenol rich fraction of *Mukia maderaspatana* by disc diffusion method (A- Control; B-25 µl/ml; C-50 µl/ml; D- 75µl/ml; E. 100 µl/ml of extract)

Table 3: Zone of inhibition of the polyphenol rich fraction of *Coscinium fenestratum* by disc diffusion method

Pathogenic organism	***Coscinium fenestratum*** **(Zone of inhibition at different concentrations)**			
	25 µl/ml (mm)	**50 µl/ml (mm)**	**75 µl/ml (mm)**	**100 µl/ ml (mm)**
Staphylococcus aureus	**7.0±1.3**	**9.2±0.6**	**11.4±1.6**	**13.6±1.4**
Pseudomonas aeruginosa	**9.3±0.4**	**12.5±0.9**	**14.3±1.1**	**16.1±0.5**
Escherichia coli	**7.8±1.3**	**10.3±0.3**	**12.7±0.6**	**15.5±0.7**
Enterococcus faecalis	**8.3±0.5**	**11.5±0.6**	**14.3±0.7**	**17.1±0.8**
	Mukia maderaspatana			
Staphylococcus aureus	**6.5±1.3**	**8.0±0.8**	**10.4±1.8**	**12.1±0.9**
Pseudomonas aeruginosa	**8.1±0.7**	**10.6±1.2**	**12.6±0.8**	**14.8±1.4**
Escherichia coli	**7.0±0.8**	**9.4±0.6**	**11.4±0.7**	**13.5±1.5**
Enterococcus faecalis	**7.3±1.6**	**10.4±0.3**	**13.5±1.8**	**15.6±1.3**



	Control	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Staphylococcus aureus,	0.568	0.453	0.395	0.324	0.268
Enterococcus faecalis	0.598	0.412	0.385	0.315	0.246
Escherichia coli	0.612	0.546	0.475	0.391	0.236
Pseudomonas aeruginosa	0.546	0.463	0.412	0.347	0.224

Figure 3: Absorbance range found in MIC of *Coscinium fenestratum*

Plant-derived phenolic compounds have gotten a lot of interest because of their multi-functionality and positive impacts on human health **[12, 13]**. The polyphenol-rich fraction of *Coscinium fenestratum* stem and *Mukia maderaspatana* leaves has been demonstrated to have remarkable antioxidant, enzyme inhibitory, and antibacterial activity in the current study. This, together with the data, suggests that the functional activity increases as the number of polyphenolic compounds increases. As a result, it can be used in the nutraceuticals industry to develop different formulations based on pharmacokinetics research to promote human health, prevent chronic diseases, extend life expectancy, or maintain the body's structure and function.

According to Harshiny *et al.,* **[14]**, *Mukia maderaspatana* leaves are a rich source of phenolic compounds with high antioxidant activity that are used as reducing agents and have also shown antibacterial activity against *Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus*, and *Salmonella typhi*. Disc diffusion was used to compare it to ceftriaxone and unconjugated nanoparticles. *Mukia maderaspatana* and *Setaria italica* in vitro bioassays form the basis for an evidence base exhibited inhibitory action in the -glucosidase inhibition assay with an IC50 of 1.1 to 1.4 g/ml, according to Kim *et al.,* **[15]**.

The *Coscinium fenestratum* plant has been utilized for treating diabetes mellitus in traditional medicine and is commonly known to be active substance with a variety of medicinal applications. The plant's stem is antimicrobial, anti-diabetic, anti-inflammatory, and anti-oxidant, and it's utilized to treat ailments **[16]**. Similarly, Compounds with anti-diabetic and antimicrobial action were identified from *Coscinium fenestratum* by Kandasamy *et al.,* **[17]** and other plant-based extracts either by directly or by synthesis of nanoparticles. **[18, 19]**.

CONCLUSION

Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the findings, indicates that as the amount of polyphenolic chemicals grows, so does the antioxidant activity. In conclusion, the present study demonstrates that the polyphenol-rich fraction of *Coscinium fenestratum* and *Mukia maderaspatana* can protect the body from oxidative stress from ROS, which may be due to the phytochemicals in the form of polyphenols that occur in the plant. So these compounds could be employed in the food and nutraceutical formulation for healthcare. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the polyphenol-rich fraction of *Coscinium fenestratum* and *Mukia maderaspatana*.

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